ESRF	**Experiment title:** Generation of hydroxyl radicals (OH•) pulses in solution by Synchrotron radiation: A new method for OH•-footprinting	**Experiment number:** LS 1413
Beamline: ID 9	**Date of experiment:** from: 8.03.99 to: 10.03.99	**Date of report:** 18.08.99
Shifts: 6	**Local contact(s):** Simone Techert; Michael Wulff	*Received at ESRF:*

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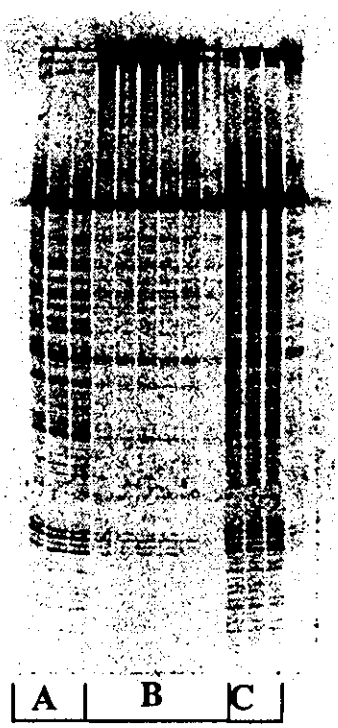
Report:

In the last beam period the binding behaviour of the RNA-polymerase to the DNA was analysed. The storage ring was running in the 4GeV (100mA) mode.
In order to determine the cleavage efficiency two different experiments have been performed:

1. Using the undulator of ID9
2. Using the wiggler of ID9 (gap: 25mm)



The results of the gel analysis are shown in fig. 1. The first three lines (A-area) are exposures of DNA using the undulator. There are well pronounced bands, indicating DNA fragments cleaved by OH•-radicals.
The cleavage bands in the C-area were produced using the wiggler at a gap of 25mm.
In difference to the undulator the bands are more pronounced but with higher background. A possible explanation therefore is that the higher photon flux of the wiggler produces also direct breaks of the DNA strand.

Fig. 1: Gel analysis of DNA and DNA-RNA-polymerase complexes after OH• radical cleavage. The ^{32}P labelled DNA is cut into fragments, which produce the cleavage pattern on the gel.

The cleavage of the DNA-RNA-polymerase complexes was done using a fast mixing device installed at the ID9. The device consists of three syringes and a quartz cuvette for the exposure. The reaction of the protein with the DNA was controlled by this syringes and several reaction times have been applied in order to trace the movement of the polymerase on the DNA template.

In fig. 2. the gel profile analysis of a exposure experiment is shown. DNA without protein is plotted as a reference (dotted lines).

Although the differences in the gel patterns induced by RNA polymerase can be seen, the exact mapping of the interaction region was not possible due to low yield of the cleavage (i.e. higher flux of radicals).

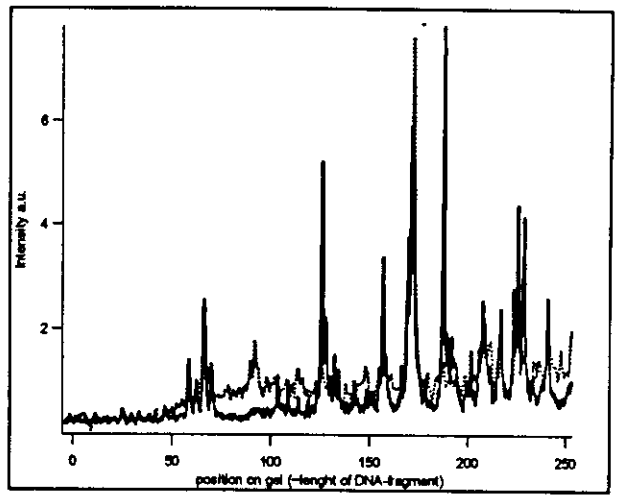
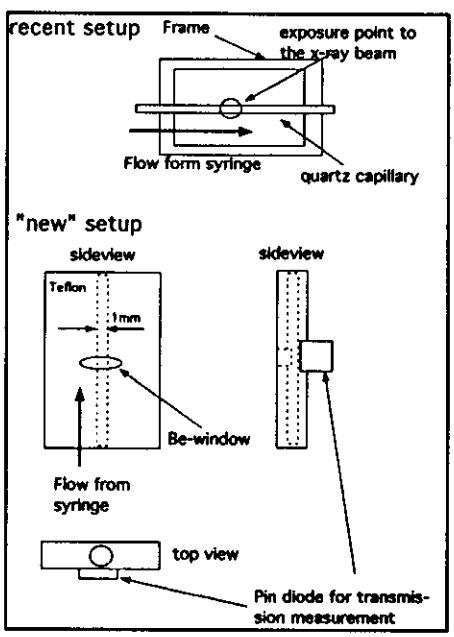


Fig. 2: Gel profile analysis of DNA-RNA-polymerase complex compared with DNA without polymase (B-area in fig. 1).

Development of a new exposure cell:



In order to gain a higher OH• radical concentration at shorter times we build up a new exposure cell. While the old cell, a quartz cuvette with thin walls (~0.1mm) shows a rather high absorption, the new flow-through cell consists of a coated Beryllium window with a better transmission. This improvement will allow us to use shorter exposure times even at lower flux conditions of the synchrotron (16-bunch etc.).

Using this set-up together with the stepping motor driven mixing device, we will be able to follow the binding reaction as well as the movement of RNA-polymerase along the DNA template in time frames of 100ms.

Fig. 3: New flow-through cell.
The quartz capillary will be changed (recent set-up) to a flow-trough cell with a Be- window.
A pin diode for transmission measurements is mounted on the backside of the cell.

Conference Contributions:

E. Zaychikov: Second joint meeting of the German Molecular Biophysics Section and the Netherlands Biophysics Society at Huenfeld (invited talk)
M. Roessle: Second joint meeting of the German Molecular Biophysics Section and the Netherlands Biophysics Society at Huenfeld (poster contribution)
H. Heumann: Talk EMBL Hamburg, International Workshop of: Potential Future Applications in Structural Biology of an X-ray FEL